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ANTIBACTERIAL ACTIVITY OF *Pluchea indica* LEAF EXTRACT WAS INCREASED AFTER BEING FERMENTED WITH *Saccharomyces cerevisiae* AND ADDED WITH ITS CELL-FREE SUPERNATANT

AKTIVITAS ANTIBAKTERI EKSTRAK DAUN Pluchea indica MENINGKAT SETELAH DIFERMENTASI DENGAN Saccharomyces cerevisiae DAN DITAMBAHKAN DENGAN CELL-FREE SUPERNATANTNYA

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ABSTRACT

Multi-Drug Resistance (MDR) is a global health problem that endangers public health and can lead to death. Therefore, novel antibacterial agents are required derived from medicinal plants. One of them is beluntas (Pluchea indica) which has high potential as an antibacterial. Fermentation or addition of cell-free supernatant of Saccharomyces cerevisiae is thought to increase the antibacterial content of an herb. This study aimed to evaluate the effect of fermentation and the addition of Cell-Free Supernatant (CFS) of S. cerevisiae to enhance the antibacterial activity of P. indica leaf extract. Fresh and dried leaves of beluntas were used in this study. The dried leaves in the form of powder were boiled at 100ºC for 45 minutes. Fresh leaves were homogenized by blending. The extract of P. indica was used for fermentation and addition of CFS of S. cerevisiae. CFS as much as 60 mL dan fermentation with a cell density of 7.53x105 CFU/mL with 100 mL of P. indica leaf extract were centrifuged at 10,000 rpm for 20 minutes. The antibacterial test method used was the Kirby-Bauer method against Escherichia coli and Staphylococcus aureus. The results showed an increase in antibacterial activity as indicated by the increasing diameter of the inhibition zone either by fermentation or addition of CFS with an inhibition zone diameter was 3.85 – 4.81 mm against E. coli and 4.63 – 5.12 mm against S. aureus. The fermented P. indica and addition of CFS were shown to be potentially developed as antibacterial agents.

Keywords: *Antibacterial, CFS, fermentation, Pluchea indica, Saccharomyces cerevisiae*

ABSTRAK

Multi Drug Resistance (MDR) merupakan masalah kesehatan global yang membahayakan kesehatan masyarakat dan dapat menyebabkan kematian. Oleh karena itu, diperlukan agen antibakteri baru yang berasal dari tanaman obat. Salah satunya adalah beluntas (Pluchea indica) yang berpotensi tinggi sebagai antibakteri. Fermentasi atau penambahan Cell-Free Supernatant (CFS) Saccharomyces cerevisiae diduga dapat meningkatkan kandungan antibakteri suatu herba. Penelitian ini bertujuan untuk mengevaluasi pengaruh fermentasi dan penambahan Cell-Free Supernatant (CFS) S. cerevisiae terhadap peningkatan aktivitas antibakteri ekstrak daun P. indica. Daun beluntas segar dan kering digunakan dalam penelitian ini. Daun kering berbentuk serbuk direbus pada suhu 100ºC selama 45 menit. Daun segar dihomogenkan dengan cara diblender. Ekstrak P. indica digunakan untuk fermentasi dan penambahan CFS S. cerevisiae. CFS sebanyak 60 mL dan fermentasi dengan kerapatan sel 7,53x105 CFU/mL dengan 100 mL ekstrak daun P. indica disentrifugasi dengan kecepatan 10.000 rpm selama 20 menit. Metode uji antibakteri yang digunakan adalah metode Kirby-Bauer terhadap Escherichia coli dan Staphylococcus aureus. Hasil penelitian menunjukkan adanya peningkatan aktivitas antibakteri yang ditunjukkan dengan bertambahnya diameter zona hambat baik dengan fermentasi maupun penambahan CFS dengan diameter zona hambat 3,85 – 4,81 mm terhadap E. coli dan 4,63 – 5,12 mm terhadap S. aureus. P. indica yang difermentasi dan penambahan CFS terbukti berpotensi dikembangkan sebagai agen antibakteri.

Kata kunci*: Antibakteri, CFS, fermentasi, Pluchea indica, Saccharomyces cerevisiae*

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INTRODUCTION

Multi-drug resistance (MDR) is a condition where bacteria are resistant to antibiotics in diseases caused by bacterial infections that can cause high mortality every year. Therefore, new antibacterial agents are needed to be developed from medicinal plants, one of which is beluntas which has high potential as an antibacterial. *Pluchea indica* or beluntas is a plant that has long been known for its benefits by the public and is quite widespread in Indonesia. According to Andarwulan et al. [1], *P. indica* leaves contain

bioactive compounds such as tannins, flavonoids, polyphenols, and essential oils that have antibacterial effects. Komala et al. [2] stated that *P. indica* leaf extract with a concentration of 3% showed inhibition against *Staphylococcus epidermidis*, which was characterized by the absence of bacterial growth. Recent research by Amilah & Ajiningrum [3] showed that beluntas leaves at a concentration of 20 mg/100 mL were able to inhibit the growth of *Mycobacterium tuberculosis*. This study provides the fact that the bioactive compounds in *P. indica* leaves can overcome bacterial infections by inhibiting their growth.

The active compounds in *Pluchea indica* that are useful in pharmaceuticals are intensively extracted directly from the plant material. However, this method is often uneconomical and shows detrimental effects on the environment after harvesting the crop. The content of compounds in plants is relatively low, so as a pharmaceutical, it eliminates the possibility of producing these secondary metabolites directly from plants in large quantities [4]. Some secondary metabolites in plants can also be produced through chemical synthesis. However, the structural and stereochemical complexity of the typical plant metabolites requires sophisticated methods for their synthesis. This method relies heavily on crude oil and processes that damage the environment. So that a more environmentally friendly compound synthesis method is needed, one of which is the synthesis with the help of microbes.

Microorganisms are widely used for the synthesis of secondary metabolites, such as alcohol, terpenoids, alkaloids, phenylpropanoids, and polyketides that act as antibacterials. *Saccharomyces cerevisiae* is a yeast that is used for production even on commercial-scale biological molecules due to their high fermentative capacity and status as safe organisms to add to food [5]. Synthesis of appropriate chemical compounds in herbal medicine using *S. cerevisiae* can contribute to increased immune activity, and the number of chemical compounds also plays an important significant role in the biotransformation of herbal medicines [6]. Thus, the synthesis of bioactive compounds with *S. cerevisiae* can effectively increase the therapeutic potential of beluntas leaves. More specifically, *S. cerevisiae* stimulates the production or conversion of the main active components of medicinal plants into their metabolites, which can enhance the therapeutic effect of herbal medicines [7].

Several studies on the synthesis using *S. cerevisiae* have been carried out. This method is effective in biological synthesis. In general, fermentation is one of the effective methods in the synthesis of compounds in plants using *S. cerevisiae* cells. However, research on synthesis with the addition of Cell-Free Supernatant (CFS) *S. cerevisiae* is still a debate, so further research is needed to determine the most optimum biological synthesis method using yeast. Therefore, this study aimed to explore the possibility of applying cell biomass and Cell-Free Supernatant (CFS) *S. cerevisiae* to increase the antibacterial activity of *P. indica* leaf extract.

METHODS

Propagation of *S. cerevisiae*. The growth medium of *S. cerevisiae* used in this study was Sabourod Dextrose Broth (SDB), by dissolving 1 g of Yeast Extract and 1 g of glucose into 100 mL of distilled water, heated, and stirred until homogeneous, then sterilized using an autoclave at 121ºC for 15 minutes. Propagation of *S. cerevisiae* by taking 10 mL of pure culture of *S. cerevisiae* from SDB media, then added to 100 mL of SDB media and then incubated at 30ºC for 24 h.

***Pluchea indica* extraction**. In this study, the extraction of *P. indica* was carried out in two ways, namely infusion and fresh extract. The infusion extract was obtained through 5 g of dried *P. indica* leaves in powder form dissolved in 100 mL of distilled water and boiled on a hotplate at 100ºC for 45 minutes. Then it was filtered using filter paper 0.45 µm, centrifuged at 5000 rpm, and the supernatant was filtered again using filter paper 0.45 µm. The fresh extract was obtained through 50 g of fresh *P. indica* leaves dissolved in 100 mL of distilled water and homogenized using a blender. Then it was filtered using a filter membrane with diameter of 0.45 µm, centrifuged at 5000 rpm, and the supernatant was filtered again using a filter membrane with diameter of 0.45 µm. The extract of *P. indica* was used for fermentation and added with CFS of *S. cerevisiae*.

Fermentation and addition of CFS using *S. cerevisiae*. Fresh cultures of *S. cerevisiae* were centrifuged at 1500 rpm for 30 min to separate cells and media. Cell-Free Supernatant (CFS) as much as 60 mL was added into 100 mL of fresh *P. indica* extract and infusion and then vortexed until homogeneous [8]. Then it was incubated in a dark place at 30ºC with variations in incubation time namely:

1. Infusion extract = The infusion extract without the addition of CFS
2. IC24 = the infusion extract with the addition of CFS incubated for 24 hours
3. IF72 = the infusion extract with fermentation incubated for 72 hours
4. Fresh extract = The fresh extract without the addition of CFS

5. SF48 = the fresh extract with fermentation incubated for 48 hours
6. SC48 = the fresh extract with addition of CFS incubated for 48 hours.

Fermentation was carried out by taking *S. cerevisiae* cells with a cell density of $7.53x10^5$ CFU/mL added into 100 mL of fresh extract and infusion, then vortexed until homogeneous [9]. The incubation results for all treatments were centrifuged at 10,000 rpm for 20 min. The pellet was discarded while the supernatant was subjected to an antibacterial test.

Antibacterial test. The test method used was the Kirby-Bauer method (disk-diffusion method). The inoculum of *E. coli* and *aureus* was measured using spectrophotometry with a wavelength of 600 nm to obtain an absorbance of 0.1, which was equivalent to 1.5 x 10^8 CFU/mL. A 100 µL of the bacterial suspension was spread aseptically onto sterile Petri dishes containing NA medium. Paper discs with size 6 mm were dripped with 70 µL of infusion extract, IC24, IF72, fresh extract, SC48, and SF48. Streptomycin 10 µg was used as a positive control and distilled water for the negative control. Paper discs that had been dripped with the treated samples were placed aseptically on the surface of the NA medium that had been inoculated with *S. aureus* and *E. coli*, then incubated at 37ºC for 24 hours. After incubation, the inhibition zone around the paper disc was observed, and the diameter was measured using a caliper. Inhibition testing was carried out in triplicates for each sample tested.

Data analysis. Analysis of the data in this study used Kolmogorov-Smirnov test statistical analysis to determine the distribution of data. Furthermore, the Analysis of Variance (ANOVA) test was carried out followed by the Tukey test with a 95% confidence level.

RESULTS AND DISCUSSION

The measurement of antibacterial activity was carried out by the agar diffusion method against *E. coli* and *S. aureus* bacteria by measuring the diameter of the inhibition zone formed around the disc. The results of the clear zone indicated the presence of bacterial inhibitory activity by the active compound *P. indica* with fermentation or addition of CFS. The results of the measurement of antibacterial activity are presented in Figure 1 and Figure 2.

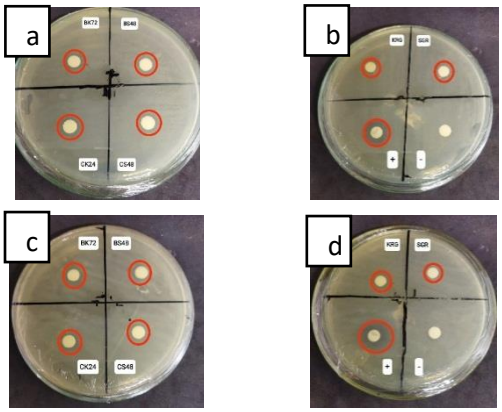

Figure 1. Inhibition zone of antibacterial activity of *P. indica* extract without and with fermentation or addition of CFS (a) with fermentation or addition of CFS against *E. coli* (b) without fermentation or addition of CFS against *E. coli* (c) with fermentation or addition of CFS against *S. aureus* (d) without fermentation or addition of CFS against *S. aureus*, (+) streptomycin, (-) distilled water.

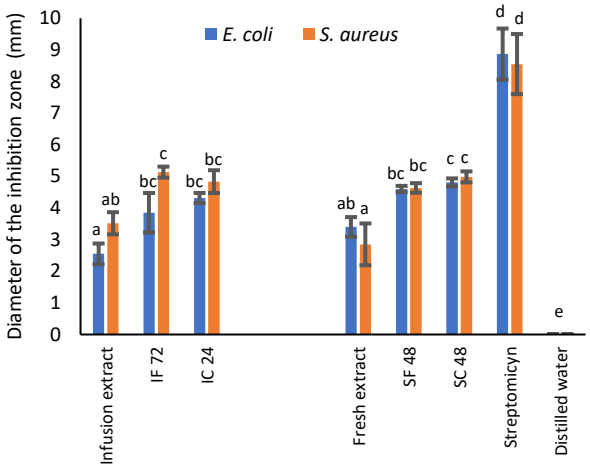

Figure 2. Diameter of the inhibition zone of *P. indica* antibacterial test without and with fermentation or addition of CFS. F72 = Infusion fermentation with 72 hours incubation, IC24 = Infusion with addition of CFS with 24 hours incubation, SF48 = Fresh extract fermentation with 48 hours incubation, SC48 = Fresh extract addition of CFS with 48 hours incubation. Different letters indicate significant differences between treatments ($p \leq 0.05$).

Active compounds in *P. indica* such as tannins, alkaloids, and flavonoids play a major role in inhibiting the growth of pathogenic bacteria, namely *E. coli* and *S. aureus.* According to Rafael et al. [10], flavonoids can inhibit the function of the cytoplasmic membrane, inhibit nucleic acid synthesis, and inhibit bacterial activity. Tannin compounds can interfere with cell permeability because of their ability to shrink cell walls or cell membranes. In the non-selectively permeable cell wall, these compounds easily penetrate the cell

wall, which will disrupt the integrity of the bacterial cell wall [11]. Alkaloids are included in the antibacterial group that can interfere the permeability of microbial cell membranes, cause damage to cell membranes and cause the release of various intracellular important components of the microbial cells, namely proteins, nucleic acids, and nucleotides [12]. The group of alkaloids in *P. indica* includes Plucheoside A, Plucheoside B, and Plucheoside E.

The antibacterial activity of *P. indica* fermentation or the addition of CFS was increased (Figure 2). It is suspected that there was an interaction between biomass and CFS of *S. cerevisiae* with *P. indica* extract. The antibacterial activity of the infusion extracts as measured by the diameter of the clear zone was 2.55 mm in *E. coli* and 3.52 mm in *S. aureus*. Meanwhile, IF72 increased by 33.7% to 3.85 mm and IC24 by 40.9% to 4.32 mm in *E. coli*. Meanwhile, in *S. aureus*, IF72 increased by 31.2% to 5.12 and IC24 by 27.1% to 4.83 mm. The fresh extract had a clear zone diameter of 3.4 mm against *E. coli* and 2.85 mm against *S. aureus. Pluchea indica* SF48 increased by 26% to 4.6 mm and SC48 by 26.5% to 4.81 mm in *E. coli*. While in *S. aureus*, SF48 increased by 40.7% to 4.63 mm and SC48 by 42.7% to 4.98 mm.

The SC48 has the greatest antibacterial activity in inhibiting the growth of *E. coli*. Meanwhile, IF72 had the greatest antibacterial activity against *S. aureus.* This shows the optimum incubation time of 48 hours to inhibit the growth of *E. coli* bacteria, while the optimum incubation time was 72 hours to inhibit the growth of *S. aureus* bacteria. It shows that the duration of fermentation influenced antibacterial activity because the longer the fermentation, the more active the microbes, which means they multiply, the more in number, so that they can break down the substrate greater [13]. Saidi et al. [14] reported that CFS from *S. cerevisiae* had an anti-biofilm effect formed by *S. aureus*. Among the various extracellular metabolites present in CFS of *S. cerevisiae*, mannoproteins and biosurfactants inhibited biofilm formation and dispersion. Various enzymes, peptides, and antibiotics contained in the CFS of *S. cerevisiae* during the biosynthesis process can inhibit pathogenic bacteria. The inhibition of the growth of *E. coli* and *S. aureus* bacteria was probably caused by the inhibition of nucleic acid synthesis by antibacterial compounds from plant extracts produced by biosynthesis and fermentation with *S. cerevisiae*, so protein synthesis was also inhibited. If protein synthesis is inhibited, it will inhibit the growth of the bacteria. These antibacterial compounds will diffuse out of the colony into the agar medium and result in the formation of a clear zone in the bacterial growth medium [15]. The antibacterial ability of *S. cerevisiae* may also be due to the production of extracellular proteases, secretion of inhibitory proteins, stimulation of immunoglobulin A [16], acquisition, and elimination of secreted toxins [17], killer toxins, sulfur dioxide etc. [18]. Killer Toxin (KT) produced by *S. cerevisiae* in the fermentation process also plays a role in inhibiting pathogenic bacteria. The KT inhibits bacteria at the early plasmodia sporogonic stage (ookinetes), causing several morphological and structural changes, thus endangering the survival of the parasite [16]. Its antimicrobial action has been attributed to the activity of glucanase, which is responsible for the hydrolysis of glucans located on the bacterial cell membrane, which are then translocated to secondary receptors on the plasma membrane causing osmotic lysis and cell death [19]. The increase in antibacterial activity in *P. indica* with the fermentation or addition of CFS may be due to the interaction of antibacterial compounds in *P. indica* with metabolites and KT produced by *S. cerevisiae*.

Saccharomyces cerevisiae significantly increased the content of antibacterial compounds, namely polyphenols [20]. Polyphenols are generally enhanced through enzymatic reactions during fermentation. As an enzyme produced by *S. cerevisiae*, glucosidase is responsible for hydrolyzing glycosidic bonds, potentially releasing phenol aglycones to increase phenolic content [20]. Fermentation with *S. cerevisiae* can increase the concentration of phenolic acids in beluntas mainly due to damage to plant cell walls by yeast which releases phenolic compounds as antibacterial compounds.

From six samples of *P. indica* without and with fermentation or addition of CFS tested, various diameters of inhibition zones were obtained. This may be due to differences in the extraction method, duration of fermentation, and the sensitivity of the test bacteria. The content of bioactive compounds can be affected by temperature. High temperatures can damage several types of these bioactive compounds [21]. Flavonoids are one of the bioactive compounds that are thought to have been damaged due to the heating process at high temperatures. The same opinion was expressed by Yuliantari et al. [21] that temperatures above 50^{o}C can cause damage to flavonoid compounds.

CONCLUSION

Antibacterial activity of *P. indica* leaf extract was increased after fermented or added with CFS of *S. cerevisiae,* indicated by the increasing diameter of the inhibition zones. The best

antibacterial activity was in SC samples which were incubated for 48 hours against *E. coli* bacteria which was 4.81 mm, and IF samples with 72 hours of incubation against *S. aureus* bacteria which was 5.12 mm.

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